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The consequences of exposure to mission-related shock waves upon cognitive potential

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: The importance of prompt identification of mTBI is crucial, since following a blast, the severe TBI soldier is easily identified (and evacuated), while the mTBI affected soldier may remain in the arena, and may be assigned duties there, that are beyond his or her reduced capabilities, in particular impairments of judgment and orientation that may result from the sustained brain concussion. One of the most important stages in this model development is to comprehend the underling mechanism (genetic, biochemical, molecular and cellular events), which in the long run will produce the cognitive and behavioral decline. We propose to determine the pathophysiologic (genetic, biochemical, molecular and cellular) mechanisms of blast over- and under-pressure waves and correlative brain injuries in animal models. Using the results from these efforts, investigators will translate and correlate the knowledge gained into a realistic patient care system capable of a standardized injury diagnosis and effective treatment and rehabilitation plan for the blast-injured patient in any stage of injury. Primary blast injuries are caused by barotraumas (either over pressurization or under pressurization relative to atmospheric pressure). Bodyarmor does not protect against these barotraumas, and from both clinical and research points of view, it is clear that once the survivor overcomes the physical consequences (to chest / abdomen / limbs / ears) of the blast injury, one of the major problems that emerges, is the cognitive, affective and behavioral changes induced by the blast exposure. We have recently developed a blast injury model for mice that resembles, as much as possible, a realistic combat blast exposure, where the outcome may vary from mild to severe brain injury. We have done this in a manner that avoids confounders such as physical injury and its consequences. Promptly following the blast, all the mice underwent a neurological assessment and some of the mice were sacrificed for a thorough pathological examination. No differences in the assessment or large pathological were found in the blasted mice, and no differences were found in the motor activity - indicating a relatively normal well-being state of the blasted mice. Then, 7 and 30 days post blast, the mices cognitive and behavioral abilities were tested using the staircase maze (restlessness/agitation), object recognition (visual memory) and Y maze (spatial memory). Other animals were evaluated using MRI for their brain integrity at the same time points. At 7 days post blast long-term cognitive and behavioral deficits and significant decreased performance were found at both 4 and 7 meters distance from the blast (2.5. and 4 PSI, respectively). At 30 days post blast, clear differences were found for both distances groups in the staircase, object recognition test, and in the 7 m group only in the Y maze test. Significant alterations were found in the magnetic resonance imaging (MRI) section of the study, we used the T1 weighted and Diffusion-weighted echo planar (DTI) images. T1 weighted images showed an increased BBBpermeability one month post-blast. DTI measures showed differences, which represent CNS abnormality in regard to water diffusivity.

15. SUBJECT TERMS EOARD, Behavior Based Prediction, Behavioral Science

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Proposal Title: "The consequences of exposure to mission-related shock waves upon cognitive potential".

Research Area: Correlating degree of injury exposure to molecular and cellular alteration from mild TBI

Collaboration: The proposed research would be collaboration between Tel Aviv University and Dr. Catherine R. Harrison, Ph.D. AFRL/HEPA

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Introduction and background:

Improvised explosive devices (IEDs) are one of the main causes for casualties among civilians and military personnel in the present war against terror. Very few studies are describing the neurological and cognitive consequences of blast injury. Most of these studies either describe humans studied in non-controlled conditions, or elegant animal models that do not resemble real-life situations, and, although some of them perform some behavioral tests, they do not extensively assess the neurocognitive outcome of blast injury and correlate it with molecular and cellular deficits.

Primary blast injuries are caused by barotraumas (either over pressurization or under pressurization relative to atmospheric pressure). Body armor does not protect against these barotraumas, and from both clinical and research points of view, it is clear that once the survivor overcomes the physical consequences (to chest / abdomen / limbs / ears) of the blast injury, one of the major problems that emerges, is the cognitive, affective and behavioral changes induced by the blast exposure.

Nowadays, traumatic brain injury caused by passively and remotely detonated explosives accounts for a larger proportion of military casualties than in other wars (Okey, 2005). Late effects of blast injury may include PTSD, mood, anxiety, and panic disorders, cognitive disturbances, as well as post-traumatic epilepsy.

We have recently developed a blast injury model for mice that resembles, as much as possible, a realistic combat blast exposure, where the outcome may vary from mild to severe brain injury. We have done this in a manner that avoids confounders such as physical injury and its consequences.

Promptly following the blast, all the mice underwent a neurological assessment and some of the mice were sacrificed for a thorough pathological examination. No differences in the assessment or large pathological were

found in the blasted mice, and no differences were found in the motor activity - indicating a relatively normal “well-being” state of the blasted mice. Then, 7 and 30 days post blast, the mice’s cognitive and behavioral abilities were tested using the staircase maze (restlessness/agitation), object recognition (visual memory) and Y maze (spatial memory). Other animals were evaluated using MRI for their brain integrity at the same time points.

At 7 days post blast long-term cognitive and behavioral deficits and significant decreased performance were found at both 4 and 7 meters distance from the blast (2.5. and 4 PSI, respectively). At 30 days post blast, clear differences were found for both distances groups in the staircase, object recognition test, and in the 7 m group only in the Y maze test.

Significant alterations were found in the magnetic resonance imaging (MRI) section of the study, we used the T1 weighted and Diffusion-weighted echo planar (DTI) images. T1 weighted images showed an increased BBB permeability one month post-blast. DTI measures showed differences, which represent CNS abnormality in regard to water diffusivity (Pick at el., manuscript in preparation).

Molecular and biochemical results:

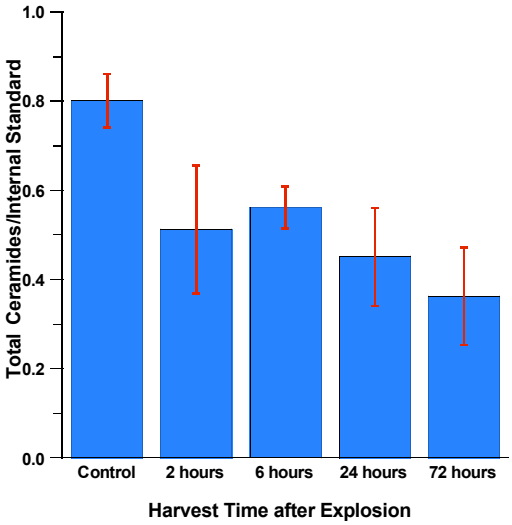
A) Lipid profiling of brains from BI-TBI mice.

Brains from control and BI-TBI were harvested at different time periods post-blast exposure. Cerebral total lipid extracts were obtained from each mouse series, purified on an SPE column and several lipid classes (including ceramides) were quantified using an ESI- Q-TOF mass spectrometer. In order to quantify the ceramide concentration an internal standard was used. The results show changes between control and BL-TBI mice, as a function of the post-blast exposure time before brain harvesting. In addition, biomarker localization was demonstrated using matrix assisted laser desorption/ ionization (MALDI) imaging mass spectrometry (IMS). This allows detailed mapping of biomolecules directly from tissue, thus providing the additional

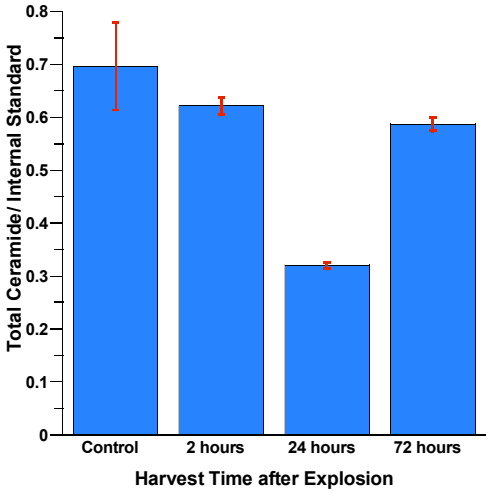
advantage of localization as well as giving an indication of the relative abundance of the various biomarkers in the different regions of the brain.

Effect of blast on ceramides

2.5 PSI (7 m)



5.5 PSI (4 m)

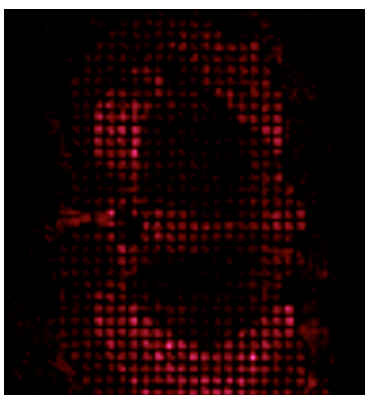


A decrease in the concentration of ceramides, bottomed out at 24 hours. Samples were spiked with an internal standard which allowed the calculation of the concentration. (Internal Standard: C12:0/d18:1 Total Ceramides: C16:0/d18:1, C18:1/d18:1, C18:0/d18:2, C18:0/d18:1, C20:0/d18:1, C24:1/d18:1, C24:0/d18:2

Effect of blast on gangliosides

Image of ganglioside GM3 d18:1 – C18:0
m/z 1179.7

Control



24h after explosion at 4m

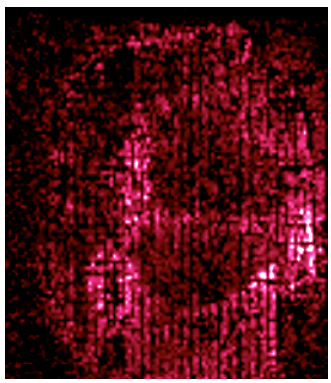
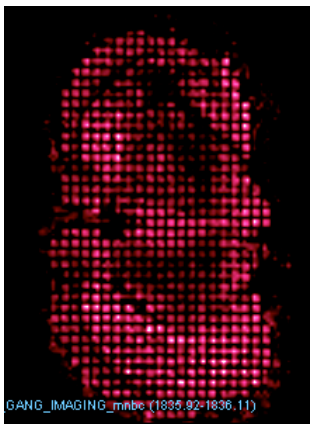
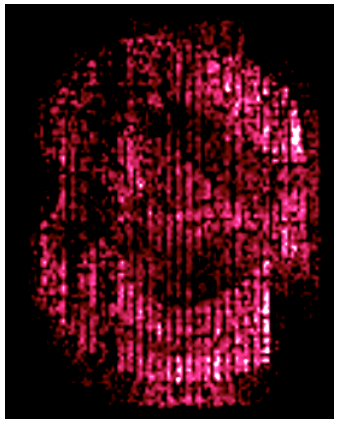


Image of Ganglioside GD1 d18:1-18:0
m/z exp = 1835.8

Control

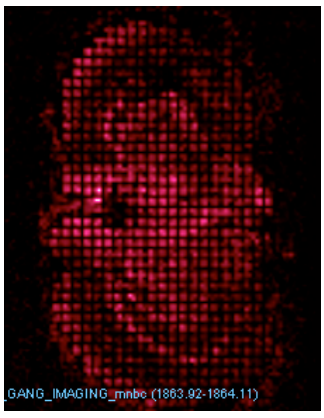


24h after explosion at 4m

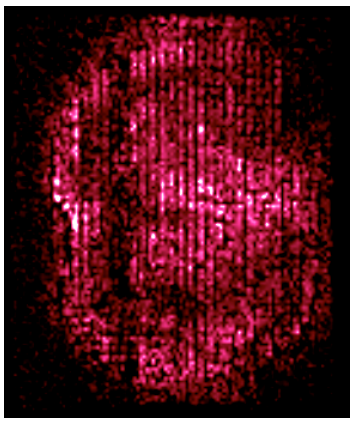


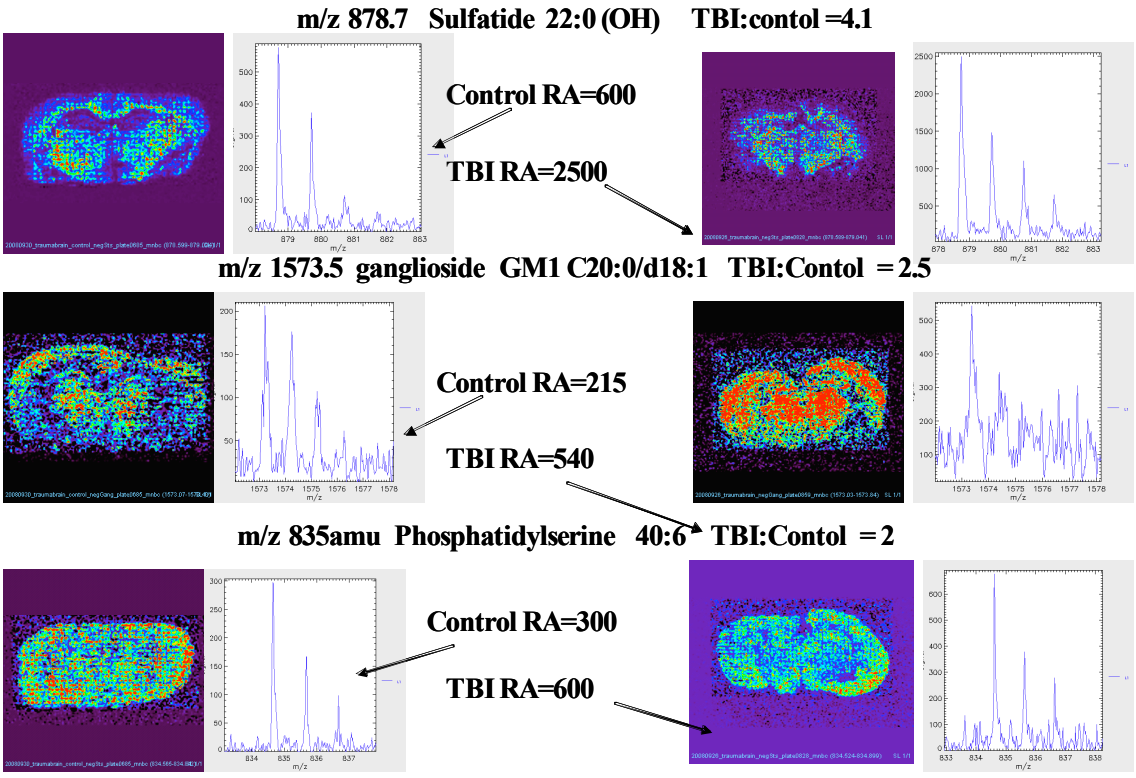
Ganglioside image of GD1 d20:1 -18:0
m/z exp = 1863.9

Control



24h after explosion at 4m





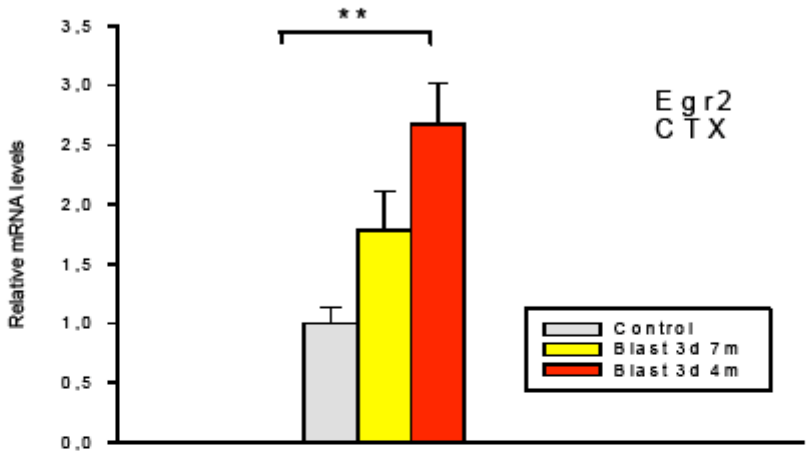
Comparison of Glycerophospholipids distribution and localization in Normal and BI -TBI brains

Data from blast exposed animals (both 2.5 and 5.5 PSI) showed a significant decrease in ceramides analyzed from a brain extract. Furthermore, imaging of cerebeoside sulfate and gangliosides from brain tissue sections of the same areas showed a marked and significant increase in all these species in the BI-TBI brains when compared with controls. Since ceramide is a membrane sphingolipid that is the scafold on which are built sphingomyelin, cerebrosides and gangliosides, the present results might reflect blast induced remodeling of the membrane organization, signal transduction alterations and myelination/demyelination balance.

B) The effect of blast on gene expression in the brain.

mRNA from neocortex and hippocampus was isolated, and transcriptional alterations studied using quantitative real-time polymerase chain reaction (PCR) and gene array analysis. Several genes were upregulated, among which were Egr2 and Dusp1, which are highly relevant to the previous results (gangliosides). Egr2 – the early growth response protein 2 (also known as krox-20) is a transcription factor. It is a positive regulator of myelination, and Dusp1 - Dual specificity protein phosphatase 1. DUSP1 may play an important role in the human cellular response to environmental stress as well as in the negative regulation of cellular proliferation. As can be seen at the attached figs the elevation of both genes was distance dependent, although significance was found only at 4 m from the explosion site.

qRT-PCR data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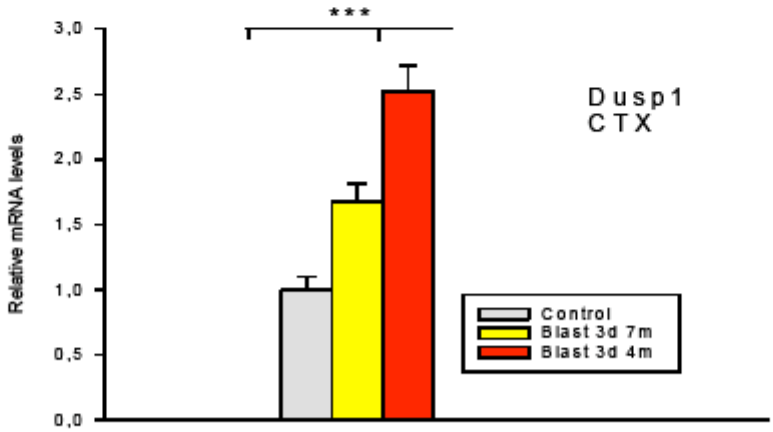
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Relative mRNA levels
Egr2
CTX
Control
Blast 3d 7m
Blast 3d 4m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3,0
2,5
2,0
1,5
1,0
0,5
0,0
Relative mRNA levels
Dusp1
CTX
Control
Blast 3d 7m
Blast 3d 4m

Conclusions

The present results are part of an ongoing multidisciplinary project investigating the mechanisms underlying blast induced brain injury and developing potential novel treatment for the survivors of blast injury. While building the model we started with designing the explosion site and the detonation device as close as possible to real combat-zone conditions. After finding the explosion extent and setting which will not kill the mice, the first animals were examined for their viability and well being; we found “normal” animals without any visible deficits. Other animals were tested for cognitive performance 7 and 30 days post blast and demonstrated long-term cognitive deficits compared with the control animals. At the same time, other groups of mice underwent an MRI scan searching for brain damage. Indeed structural damage and deficits in the DTI exposition were seen all over the brain.

In addition to all the above we found that blast exposed animals showed a significant decrease in ceramides analyzed from a brain extract. Furthermore, imaging of glycolipids, cerebeoside sulfate and Gangliosides from brain tissue sections of the same areas showed a marked and significant increase in all these species in the BI-TBI brains when compared with controls. Since Ceramide is a membrane sphingolipid that is the scafold on which are built sphingomyelin, cerebrosides and gangliosides, the present results may reflect blast induced remodeling of the membrane organization and signal transduction alterations. The results point to myelination/demyelination balance as the key step of the axonal damage. Thus, our future research will address these intriguing issues. Our findings will

be used as baseline for developing potential therapeutics, which are already under research in the lab.